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New record of *Melanophryniscus biancae* Bornschein, Baldo, Pie, Firkowski, Ribeiro & Corrêa, 2015 (Anura, Bufonidae) from Paraná, Brazil, with comments on its phytotelm-breeding ecology

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Abstract

We report *Melanophryniscus biancae* Baldo, Pie, Firkowski, Ribeiro & Corrêa, 2015 from Araçatuba, Tijucas do Sul, Paraná, Brazil at 1,615–1,645 m altitude, the first record from the state of Paraná. On 8 January 2018 we heard individuals calling during the day in the leaves of *Paepalanthus planifolius* (Bong.) Körn. where eggs, tadpoles, and three adults were collected. This species was recently described from two upland marshes (1,310–1,465 m altitude) in northeastern Santa Catarina, where it was found at night breeding in water tanks of *Eriocaulon ligulatum* (Vell.) L.B.Sm. Included here are new data on this species' habitat, activity period, plant species used for oviposition, and the first female measurements.

Keywords

Conservation, diurnal activity, grasslands, *Paepalanthus planifolius*, Refúgio Vegetacional, Serra do Mar.

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Introduction

The genus *Melanophryniscus* Gallardo, 1961 (Anura, Bufonidae) includes 29 species distributed throughout South America, from southern Bolivia to the northern half of Argentina, Uruguay, Paraguay, and southern Brazil (Frost 2019). Species belonging to this genus are small (snout–vent length [SVL] = 12.8–41.2 mm; Steinbach-Padilha 2008; Bidau et al. 2011) and present a wide range of aposematic coloration, especially on the ventral side of the body (Hantak et al. 2013). *Melanophryniscus* shows three types of oviposition strategies, namely breeding in temporary ponds, small temporary streams, and micro-waterbodies (Baldo et al. 2014). Oviposition in micro-waterbodies is represented by breeding in phytotelmata, a strategy described for *M. alipioi* Langone, Segalla, Bornschein & de Sá, 2008 (Langone et al. 2008). This reproductive mode was also described for a second species, *M. vilavelhensis* Steinbach-Padilha, 2008 (Steinbach-Padilha 2008). More recently, three

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new species were described, all of them reproducing in phytotelmata, namely *M. biancae* Bornschein, Baldo, Pie, Firkowski, Ribeiro & Corrêa, 2015, *M. milanoi* Baldo, Bornschein, Pie, Firkowski, Ribeiro & Belmonte-Lopes, 2015, and *M. xanthostomus* Baldo, Bornschein, Pie, Ribeiro, Firkowski & Morato, 2015 (Bornschein et al. 2015). These five species represent all the phytotelma-breeding *Melanophryniscus*. Another peculiarity of these species is their restricted geographical distribution, from a single locality to few localities in the states of Paraná and Santa Catarina, southern Brazil (Langone et al. 2008; Steinbach-Padilha 2008; Bornschein et al. 2015).

Melanophryniscus biancae was described from marshes in two places between 1,310 and 1,465 m above sea level (a.s.l.) in the Serra do Quiriri, municipality of Garuva, northeastern Santa Catarina (Bornschein et al. 2015). Only males were found at night, calling in water tanks on the axils of living leaves of *Eriocaulon ligulatum* (Vell.) L.B.Sm. (Eriocaulaceae; Bornschein et al. 2015). In this study, we report the first record of *M. biancae* in Paraná as well as new ecological data, including its habitat, activity period, and plant species used for oviposition. Measurements of a female individual are reported for the first time.

Methods

We searched both during the day and night for populations of *Melanophryniscus biancae* in grassland in mountains of Paraná state. Individuals were detected by the sound of their calls and some were located for collection. We also look for adult individuals, eggs, and tadpoles on plants of the family Eriocaulaceae. Eggs were counted in the plants' water tanks without the leaves being touched to avoid water flow and egg exposure to desiccation. Geographical coordinates were recorded using a GPS GARMIN etrex 10 receiver (datum: WGS84) and the altitude was found using Google Earth Pro v. 7.1.4.1529, as by Bornschein et al. (2016). Vegetation of the studied sites was classified according to the Brazilian vegetation classification system (Veloso et al. 1991).

Collection permits for this study were issued by Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio) through Sistema de Autorização e Informação em Biodiversidade (SISBIO), permit number #55918-1. Specimens were anaesthetized and euthanized in 2% lidocaine chlorhydrate, fixed in 10% formalin, and preserved in 70% ethanol solution and were deposited at Museu de História Natural Capão da Imbuia (MHNCI), Curitiba, Paraná state. We sexed as males those specimens that were found vocalizing and had (under magnification) nuptial pads on fingers I–III (Bornschein et al. 2015). Likewise, we sexed as female the single specimen without nuptial pads that was not vocalizing (Bornschein et al. 2015).

Results

New records. Brazil, Paraná, municipality of Tijucas do Sul, Serra do Araçatuba (25°54′05″ S, 048°59′44″ W; 1,615–1,645 m a.s.l.; Figs 1, 2), coll. by Marcos R. Bornschein, Luiz F. Ribeiro, and Júnior Nadaline, 8 January 2018; 2 adult males (MHNCI 11049, 11050; SVL = 12.7 mm and 12.3 mm, respectively; Fig. 3B, C); 1 adult female (MHNCI 11051; SVL = 15.3 mm; Fig. 3A).

We heard *Melanophryniscus biancae* during daytime, from 11:45 h to 15:30 h, calling in marshes (Formação Pioneira de Influência Fluvial) and grasslands with soggy soil (Estepe Gramíneo Lenhosa). Both habitats had Apiaceae (*Eryngium* sp.), Apocynaceae, Asteraceae (e.g. *Baccharis* sp.), Cyperaceae, Droseraceae (*Drosera* sp.), Eriocaulaceae (*Paepalanthus planifolius* (Bong.) Körn.), Lentibulariaceae (*Genlisea* sp., *Utricularia* spp.), Melastomataceae, Orchidaceae (*Habenaria* sp.), Poaceae (e.g. *Chusquea pinifolia* (Nees) Nees), Xyridaceae (*Xyris* spp.), ferns (e.g. *Blechnum* sp.), moss, and lichens (*Cladonia* sp.). Conditions were foggy (100% humidity; Fig. 2A) and the air temperature was 19.0–20.5 °C on the herbaceous vegetation to a constant 17.5 °C at the base of the vegetation. We collected three individuals (Fig. 3), distant from one another; two were males, which were found in the water tanks of the axils of live leaves of *P. planifolius*, and one was female, which was walking on live leaves of the same plant species. We found eggs and tadpoles of various growth stages (Fig. 4) in water tanks of the axils of live leaves of *P. planifolius* (Fig. 5), with 0–21 eggs on 10 plants ($\bar{x}$ 8.1 eggs; SD 6.30 eggs; Table 1) and 1–4 eggs in 47 water tanks ($\bar{x}$ 1.5 egg; SD 0.69 egg; Table 1; Fig. 5A). On 25 August 2018 we returned to Serra do Araçatuba during a sunny day (air temperature was 13.8 °C; Fig. 2B) and failed to find the species.

On 9–11 November 2018 we have searched for *M. biancae* 74 km at the north of Serra do Araçatuba in the grasslands (Refúgio Vegetacional) of Serra dos Órgãos, Paraná, between altitudes of 1,425–1,880 m a.s.l. We have concentrated the searches at a place called "Abrigo 2" (25°15′11″ S, 048°49′00″ W; 1,600 m a.s.l.), municipality of Antonina. It rained continuously during the first two days, but the last day was sunny. The daily temperature varied from 15.5–17.3 °C. During the night, the temperature dropped to a minimum of 13 °C. We did not find adult specimens, eggs, or tadpoles of *M. biancae* in Eriocaulaceae plants, including *P. planifolius*.

Identification. There are only five phytotelm-breeding *Melanophryniscus* described so far, namely *M. alipioi*, *M. biancae*, *M. milanoi*, *M. vilavelhensis*, and *M. xanthostomus* (Langone et al. 2008; Steinbach-Padilha 2008; Bornschein et al. 2015). Some of the species have predominantly black and red ventral surfaces (*M. alipioi*, *M. milanoi*, *M. vilavelhensis*, and *M. xanthostomus*; Langone et al. 2008, Bornschein et al. 2015), while *M. biancae* has predominantly yellow ventral surface, with only a reduced tinge of red and small black dots (Bornschein et al. 2015). Our collected specimens were

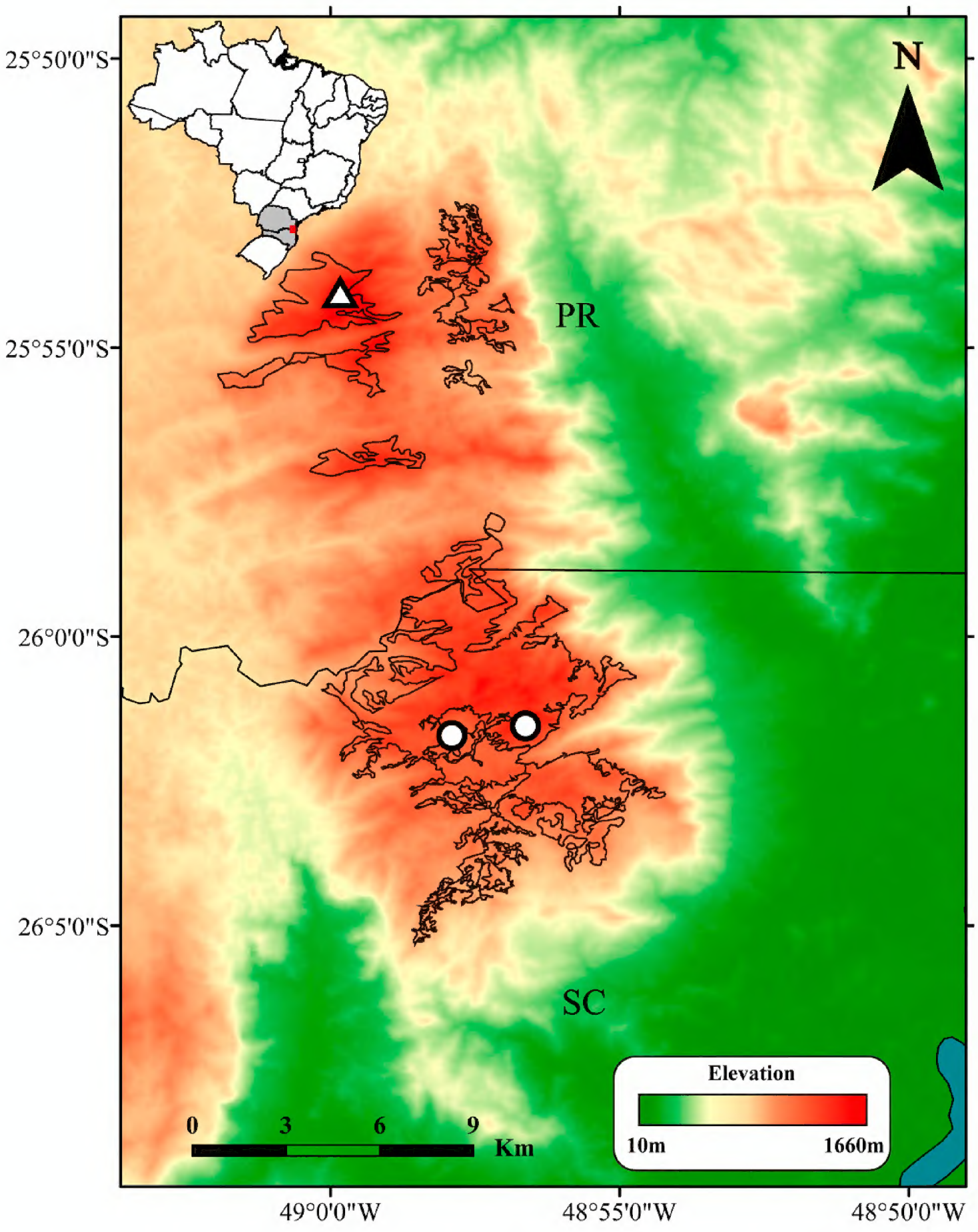

Figure 1. Geographic distribution of *Melanophryniscus biancae*, showing the new record in Paraná (PR; white triangle) and previous records (white circles) from the literature (Bornschein et al. 2015), in Santa Catarina (SC), southern Brazil. The black polygons represent the extent of occurrence of the species estimated by Bornschein et al. (2015), but hitherto unpublished.

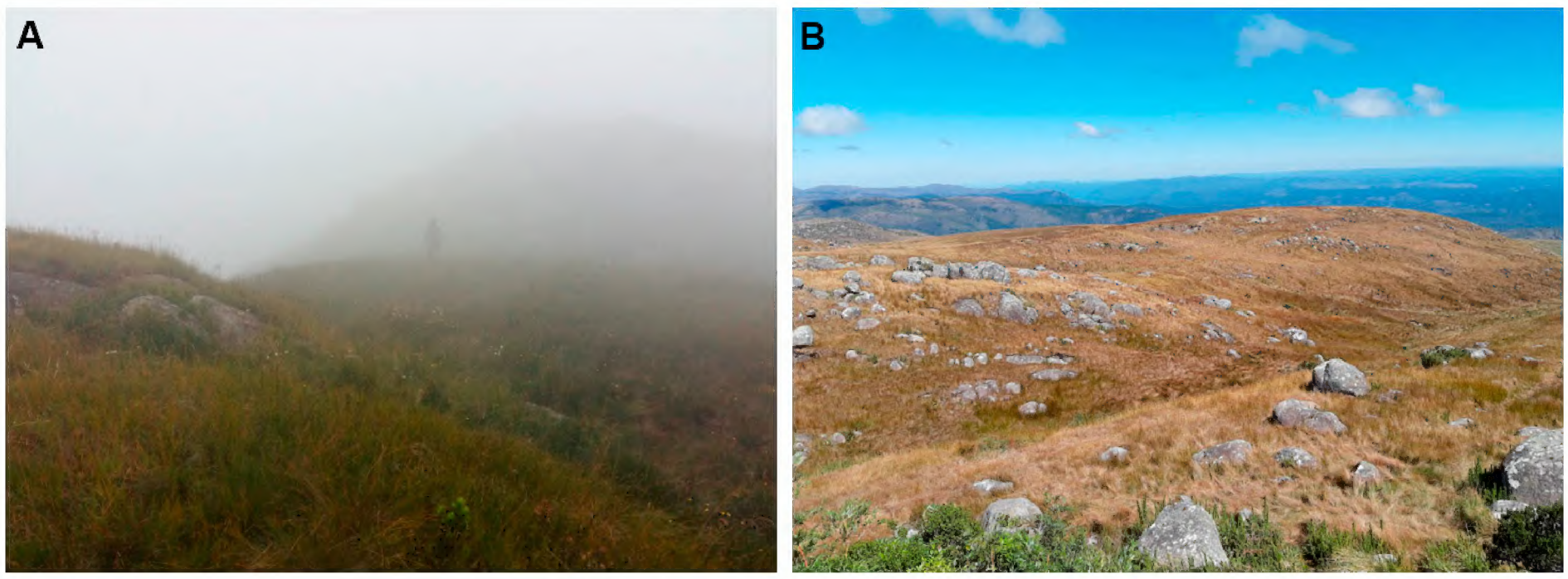

Figure 2. Locality of record of *Melanophryniscus biancae* in the Serra do Araçatuba, municipality of Tijucas do Sul, Paraná, southern Brazil. **A.** Serra do Araçatuba in a day when the species was recorded (8 January 2018). **B.** Serra do Araçatuba in a day without capture of the species (25 August 2018).

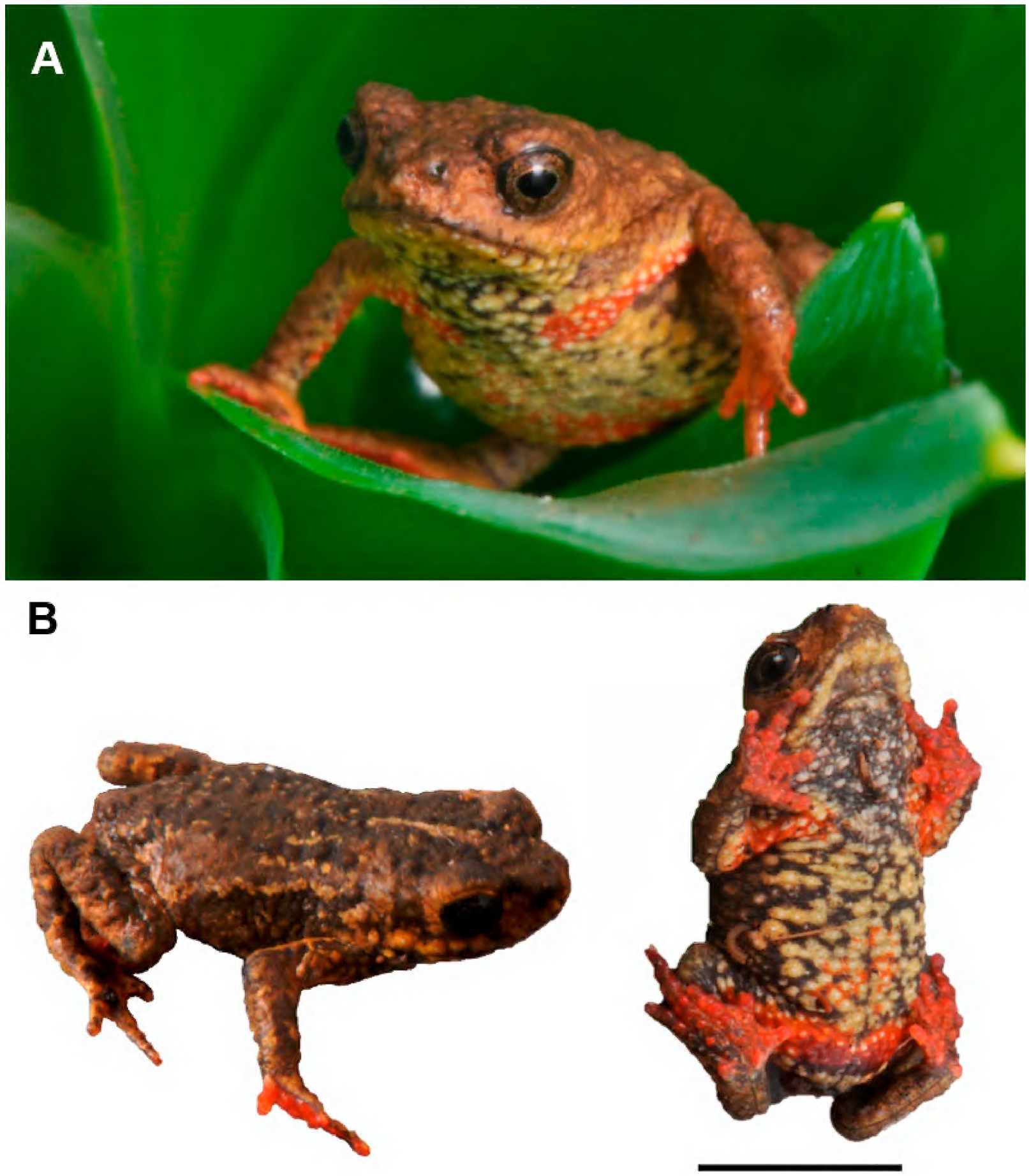

Figure 3. *Melanophryniscus biancae* collected in the Serra do Araçatuba, municipality of Tijucas do Sul, Paraná, southern Brazil. A. Female (MHNCI 11051). **B.** Male MHNCI 11049, in lateral and ventral view. **C.** Male MHNCI 11050, in lateral and ventral view. Abbreviation: MHNCI = Museu de História Natural Capão da Imbuia, Curitiba, Paraná. Scale bar = 5 mm.

Figure 4. Eggs and tadpoles of *Melanophryniscus biancae* in distinct stages of growth collected on 8 January 2018 in *Paepalanthus planifolius* leaves at Serra do Araçatuba, municipality of Tijucas do Sul, Paraná, southern Brazil.

phytotelm-breeding and matched the coloration of the type series of *M. biancae* perfectly, having a brown dorsum and yellow ventral surface with limited red area, a few black patches and dots (compare Fig. 3 with Bornschein et al. 2015: fig. 3). Additionally, our specimens were smaller (SVL = 12.3–15.3 mm) than *M. biancae* (SVL = 12.9–13.8 mm; Bornschein et al. 2015) and *M. vilavelhensis* (SVL = 12.8–17.2 mm; Steinbach-Padilha 2008), while other phytotelm-breeding species are larger (*M. milanoi* = SVL 17.6–23.7 mm and *M. xanthostomus* = SVL 18.1–21.5 mm, Bornschein et al. 2015; *M. alipioi* = SVL 19.4–25.6 mm, Langone et al. 2008). Furthermore, our specimens were found in non-forest habitats, as are *M. biancae*, *M. milanoi*, and *M. vilavelhensis* (Langone et al. 2008; Steinbach-Padilha 2008; Bornschein et al. 2015), while *M. milanoi* and *M. xanthostomus* are forest species (Bornschein et al. 2015).

Discussion

Our record from Serra do Araçatuba represents the first occurrence of *Melanophryniscus biancae* in Paraná. However, its presence at this locality was predicted based on habitat similarity and relative proximity to the type locality of the species (Bornschein et al. 2015). This

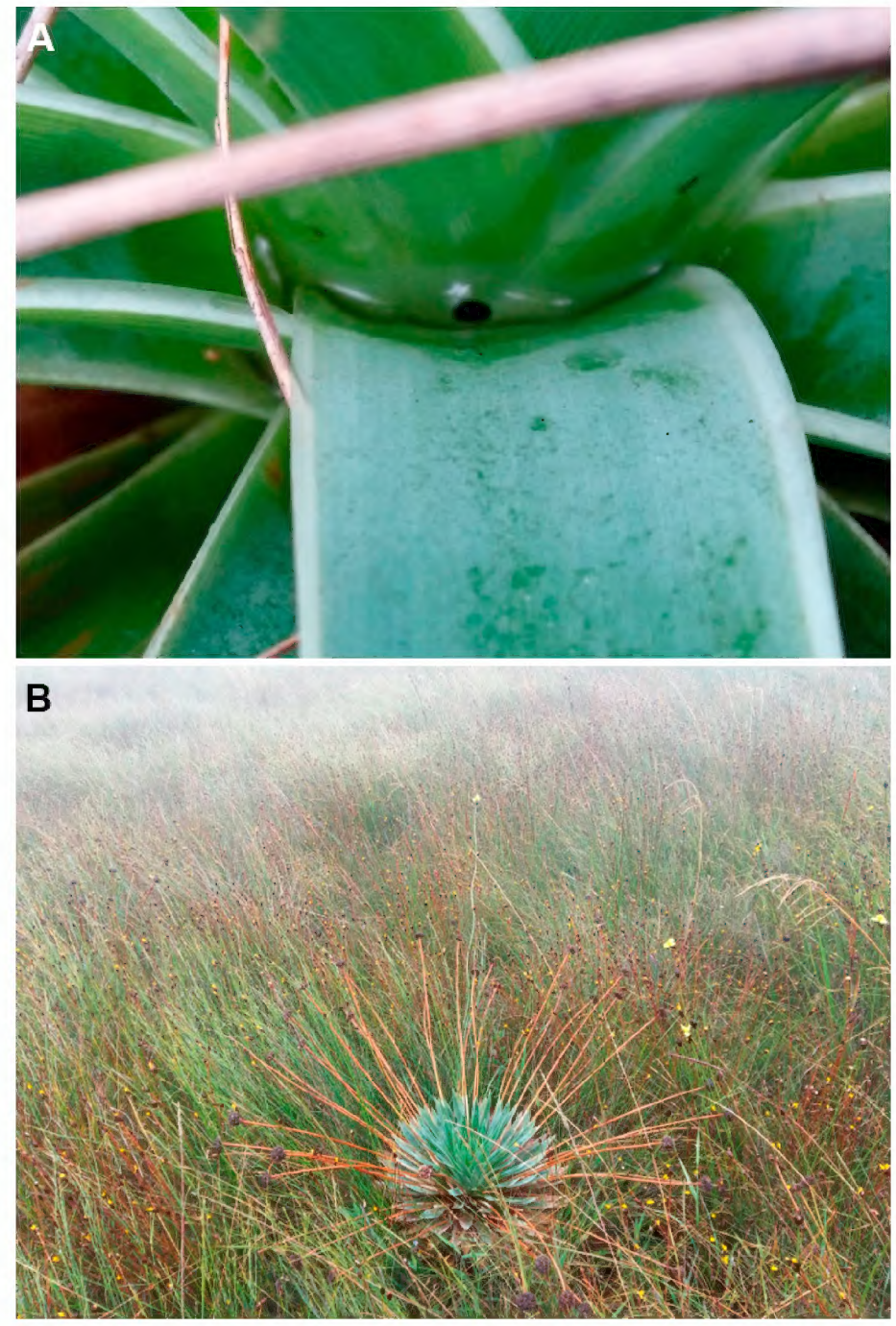

Figure 5. A. Water tank in the axil of live leaves of *Paepalanthus planifolius*, with one egg. **B.** *Paepalanthus planifolius* (foreground, center) in a grassland with soggy soil in the Serra do Araçatuba, municipality of Tijucas do Sul, Paraná, southern Brazil.

new record is 14.3 km away in a straight line from the type locality and was included in one of the polygons in estimating this species' extent of occurrence, which corresponded to an area of only 4.742 ha (Bornschein et al. 2015). Due to the small extent of occurrence and habitat suppression and degradation, Bornschein et al. (2015) proposed that *M. biancae* is Endangered under IUCN (2012) criteria. Our new record confirms this assessment. A fast and intense invasion of exotic *Pinus* spp. is underway in the Serra do Araçatuba. The land is frequently burned for grazing by livestock, which can

Table 1. Number of eggs of *Melanophryniscus biancae* counted per water tank (separated by "/") in each axil of live leaves of *Paepalanthus planifolius* in the Serra do Araçatuba, municipality of Tijucas do Sul, Paraná, southern Brazil, on 8 January 2018.

Plant individual	No. of eggs per tank	Observation
1	4/1/1/1/2/1/1	After collecting the plant, we found that there was a total of 12 eggs. Tadpoles were present.
2	2/1/2/1/1/2/2	After collecting the plant, we found additional water tanks with eggs, totaling 21 eggs. Tadpoles were present.
3	2/2/2/1/1/1/2/1/1/1	
4	3/2/2	
5	3/1/2/1	
6	2/1/2/2	There were some dead eggs covered by fungi.
7	2/1/1/1/1/1/1/1	
8	—	There were only two tadpoles in one water tank.
9	1	
10	1/1/2	There was one tadpole in one water tank.

be critical for *M. biancae*, which requires plants of the family Eriocaulaceae for reproduction (Bornschein et al. 2015; this study).

This study reports for the first time oviposition of *M. biancae* in *Paepalanthus planifolius* instead of *Eriocaulon ligulatum*, as previously reported by Bornschein et al. (2015). The presence of eggs and tadpoles at various stages of development in the same water tanks in the axillae of the plants, which was also observed by Bornschein et al. (2015), reveals the importance of each plant in consecutive cycles of explosive reproduction.

Daytime activities for *M. biancae*, as well as its occurrence in grasslands (Estepe Gramíneo Lenhosa), have also been documented here for the first time. However, we cannot confirm that diurnal activities are usual for the species or only related to extremely wet and shady days. Moreover, we cannot confirm that the species is widespread in the grasslands or limited to the ones with soggy soil and the presence of Eriocaulaceae. Despite these uncertainties, this new information indicates higher ecological plasticity than previously documented for this species (Bornschein et al. 2015).

Paepalanthus planifolius is more widely distributed in the grasslands along the Serra do Mar than *Eriocaulon ligulatum* (MRB pers. obs.), which is recorded in association with vast grassland areas called Estepe Gramíneo Lenhosa (sensu Veloso et al. 1991; see Bornschein et al. 2015, Pie et al. 2017). In Paraná's portion of the Serra do Mar, Estepe Gramíneo Lenhosa is restricted to the Serra do Araçatuba (MRB pers. obs.), where *M. biancae* was found. The other grassland areas along the mountains of the Serra do Mar within Paraná state are small patches surrounded by forests and are called Refúgio Vegetacional (sensu Veloso et al. 1991; see Pie et al. 2017). The presence of *P. planifolius* in Refúgios Vegetacionais would suggest a possible overlap with the geographical distribution of *M. biancae*. However, we have not found *M. biancae* in the Serra dos Órgãos, despite the presence of *P. planifolius* and our intensive three-day fieldwork under very moist conditions. We confirm that the fieldwork in this locality was made under the appropriate climatic conditions for the reproduction of *Melanophryniscus* because we heard *M. alipioi* calling in bromeliads during all three days. Additionally, *M. biancae* reproduces in wet periods, with temperature ranging from 13 to 20.5 °C (Bornschein et al. 2015; this study), a similar condition to those reported for Serra dos Órgãos (13–17.3 °C; see above).

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Authors' Contributions

JR, LFR, LT, FSV and MRB participated on fieldwork and data collection; JR, MRB and LFR conducted the analysis; JR and MRB wrote the manuscript; MRB and LFR took photographs. JR, LT, FSV, MRB and LFR revised the manuscript.

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